FUNGI IN AND NEAR STREAMS CARRYING ACID MINE-DRAINAGE[1]

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Abstract. From streams carrying acid mine-drainage and from adjacent soils in 3 areas in Ohio and West Virginia, 189 species of fungi, including yeast like as well as filamentous types, were isolated using agar pour plates and shaken flask cultures. The more important members of this population were members of the Fungi Imperfecti, 25% of these yeast like and the remainder filamentous fungi. Species with phialidic conidium production represented 80% of the filamentous fungi. Larger numbers of species and colonies were recovered during the mid-autumn sampling period.

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Continuing studies of the biology of acid mine-drainage carrying streams in the soft coal producing regions of Ohio and West Virginia by units of the organization then known as the Robert A. Taft Sanitary Engineering Center, and the Ohio Basin Region of the Federal Water Pollution Control Administration, indicated a need for information about fungal populations of this habitat. A report on the studies made in 1964 and 1965 was published by Cooke (1966). In developing a catalogue of the fungi of Ohio in streams carrying acid-mine drainage, it seems appropriate that the species of fungi, previously indicated only by total numbers for a habitat, a location, a date, or the project, be listed.

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METHODS

Sample locations for the Ohio portion of the study were selected by the writer, who made all Ohio collections with the help of his wife. Personnel from the Wheeling, W. Va., and the Elkins, W. Va., laboratories assisted in choosing sites for the West Virginia samples. The writer made all initial field collections and field personnel took the second and third sets using bus express for overnight shipment to Cincinnati. Data on pH, total Fe in ppm, Fe^{++} in the presence of Fe^{+++} in ppm, and number of species and colonies of fungi in each sample on each sampling date were given in Cooke (1966). Techniques of obtaining this information were given briefly in that report.

Sample Locations — Ohio Series (O)

1. Sandy Run at King Hollow Bridge, off SR-278, Vinton Co., Ohio
2. Sandy Run below mine at mouth of Coal Hollow, Vinton Co., Ohio.
3. Sandy Run above mine at mouth of Coal Hollow, Vinton Co., Ohio.
4. Sunday Creek at junction of Township Road 291 and SR-13, Perry Co., Ohio.
5. Sunday Creek near Hemlock above bridge on SR-165, Perry Co., Ohio.
6. Bricked in spring on hillside berm of SR-216 near Murray City, Hocking Co., Ohio.
7. Creek from spring in ochre "cave", east of New Straitsville, along SR-216, Hocking Co., Ohio.
8. Crumley's Farm Creek, off US-33, near Groveport, Franklin Co., Ohio.
9. Ash Cave Falls, Ash Cave State Park, Hocking Co., Ohio.

9a. Wheeling Creek, Belmont Co., Ohio, just above Belmont Co. road No. 10, bridge below active coal mine.

Sample Locations — Morgantown Series (M)

1. Fifty yards above mouth of Scott's Run, of W. Va. Rte. 100, Monongalia Co.
2. Decker's Creek below power plant, about one mile above mouth, Morgantown.
3. Booth Creek, 2.1 mi. above Weirton Steel Co. coal mine preparation plant.
4. Booth Creek at Weirton Steel Co. coal mine preparation plant (abandoned), between gob piles and settling basin.
5. Lake Lynn on Cheat River at boat docks below State Park Lodge.
6. Near mouth of Robinson Run, 1.7 mi. n. of US-19 on right side of road.
7. Monongahela River, west bank, 2.5 mi. n. of US-19 bridge below Morgantown.
8. Monongahela River, east bank, 0.5 mi. S. of Morgantown Dam.

Sample Locations — Elkins Series (E)

1. Station R–1, near mouth of Roaring Creek, 100 ft. above bridge.
2. Station G–1, near mouth of Grassy Run, at old railroad bridge.
3. Station GT–6–1, Norton Mine # 15 drainway.
4. Station RT–5–3, Pond on White's Run, top of strip pit.
5. Station R–3, Roaring Creek at Coalton.
6. Station TR–6–17, Kittel's Run, including samples of material from aluminum and iron "travertine" deposits built up on leaf litter at springs.
7. Station R–C Special (R–9), Roaring Creek.
8. Station RT–6 Basin—Pond in strip pit by Coalton Dump.
9. Station RT–10–1. Brooks Run, tributary of Roaring Creek.

In table 1, habitats are given for each species but no attempt has been made to indicate the date or dates on which a species was recovered, and the locations have been lumped for each area. The three basic areas sampled were: Ohio, including stations on the Hocking River and upper Racoon Creek; West Virginia, including the Morgantown area, Monongalia Co., with stations in the Monongahela River basin; the Elkins area, Randolph Co., W. Va., with stations in the Cheat River basin. For each basic area 8 or 9 locations were chosen as indicated above. At each location 4 habitats were sampled: flowing water (W) in the stream in which spores were carried passively, or colonies of fungi could be actively growing; sediments (S) on the bottom of the stream at arm's length from the shore from which stream water could not be excluded but in which fungal colonies could be present as members of the benthos community; stream bank (B) soil taken at the interface of the stream and the bank so that the habitat was always affected by the stream water; and upland (U) soil usually taken above the obvious high-water mark where the habitat was rarely affected by the stream water. (see table 1).

Pour plates were prepared as in Cooke (1963). For these, two media were used in parallel: neopeptone-dextrose-rose bengal-Aureomycin, and neopeptone-dextrose-Aureomycin agars, the latter acidified to approximately pH 5.0. The third and fourth media were YNB–1% dextrose, and YNB–20% dextrose broths, prepared at room temperature and filter sterilized according to Cooke (1963). More discrete colonies appeared in the medium including rose bengal, and yeasts grew better in broth media in shaken culture. Elements of the total population, so far as it was obtained, appeared on each medium but no single medium gave a complete picture of either yeasts or filamentous fungi. In a fifth technique, Oomycetes were obtained only when portions of samples were incubated with hemp seeds (Cooke, 1963). Species were identified by the author based on either his interpretation of monographic or mycologistic treatments, or by comparison with known strains or subcultures.

TABLE 1

Systematic list of Fungi Recovered from Acid Mine Drainage Sites in Ohio and West Virginia.

Species	Total	No. of Isolations†		
		Ohio Samples*	West Virginia	
			Morgantown Samples*	Elkins Samples*
Eumycota	2827			
Mastigomycotina				
Oomycetes	11			
Saprolegniales				
Saprolegniaceae				
Achlya spp.**	6	B–1,U–1.	W–1,S–1,B–2.	
Aphanomyces sp.**	1	W–1.		
Dictyuchus sp.	1		S–1.	
Saprolegnia spp.	3		W–2,B–1.	
Zygomycotina				
Zygomycetes	252			
Mucorales				
Mucoraceae				
Absidia cylindrospora	1	S–1.		
Absidia glauca	3	S–1,B–1.	S–1.	
Actinomucor elegans	1	S–1.		
Gongronella butleri	5	S–1,U–1.		S–1,B–2.
Mucor spp.**	102	34	28	40
Mucor alternans	2	S–1.		U–1.
Mucor hiemalis	66	34	28	S–1,B–2,U–1.

TABLE 1. *Continued.*

Species	Total	No. of Isolations†		
		Ohio Samples*	West Virginia	
			Morgantown Samples*	Elkins Samples*
Mucor racemosus	2		B-1.	U-1.
Rhizopus spp.	20	W-1,S-5,B-5.	S-1,B-1.	W-1,B-2,U-4.
Rhizopus arrhizus	1	W-1.		
Rhizopus chinensis Series	3	W-1,U-1.		B-1.
Zygorhynchus moelleri	36	W-1,S-6,B-6,U-5.	W-1,S-2,B-4,U-3.	S-2,B-2,U-4.
Mortierellaceae				
Mortierella angulisporus	18	W-1,S-2,B-2,U-1.	W-2,S-2,B-1,U-3.	B-1,S-1,U-2.
Cunninghamellaceae				
Cunninghamella elegans	1	S-1.		
Piptocephalidaceae				
Syncephalastrum racemosum	1	1		
Ascomycolina	21			
Saccharomycetaceae				
Endomycopsis burtonii—see *Trichosporon behrendii*				
Hansenula saturnus	1	S-1.		
Pichia guilliermondii	7	S-1,B-1,U-1.	B-2.	B-1,U-1.
Saccharomyces sp.	2	B-2.		
Saccharomyces cerevisiae	10	W-1,B-2.	W-1,S-2.	S-2,B-1,U-1.
Saccharomyces rosei	1	B-1.		
Plectomycetes				
Eurotiales				
Melanosporaceae				
Chaetomium globosum	1		W-1.	
Sordariaceae				
Pleosporales				
Sporormiaceae				
Preussia multispora	1	B-1.		
*Basidiomycotina***				
Teliomycetes				
Ustilaginales				
Rhodotorula glutinis				
Deuteromycotina	2542			
Coelomycetes				
Sphaeropsidales	152			
Sphaeropsidaceae				
Chaetomella sp.	3		B-1,U-2.	
Coniothyrium sp.	4	S-1, 1.		
Coniothyrium fuckelii	1	1		
Cytospora sp.	1	1		
Phoma spp.	108	+30	+24	+30
Phoma herbarum	31	W-4,S-5,B-7,U-2.	W-2,S-3,B-4,U-2.	W-2.
Selenophoma sp.	1	1		
Septoria sp.	1		1	
Sphaeronema spinella	1	1		
Blastomycetes				
Cryptococcales				
Cryptococcaceae				
Candida curvata	26	W-1,S-3,B-3,U-2.	S-2,B-2,U-1.	W-2,S-3,B-3,U-4.
Candida guilliermondii	15	W-2,S-1,B-1.	W-1,S-3,B-3,U-2.	S-1,U-1.
Candida humicola	14	S-3,B-2,U-1.		S-2,B-4,U-2.
Candida intermedia	6	U-1.	B-3.	B-1,U-1.
Candida krusei	37	S-4,B-6,U-4.	W-3,S-6,B-4,U-3.	W-3,S-1,B-2,U-1.
Candida mycoderma	7	B-1,U-1.	S-1.	S-1,B-1,U-2.
Candida parapsilosis	42	W-3,S-4,B-3,U-1.	W-2,S-6,B-4,U-3.	W-3,S-6,B-4,U-3.
Candida pseudotropicalis	1	B-1.		
Candida rugosa	2	B-2.		
Candida scottii	2		S-1	B-1.
Candida solani	4		B-1.	W-1,B-1,U-1.
Candida tropicalis	4	B-1,U-1.	U-1.	S-1.

TABLE 1. *Continued.*

Species	Total	No. of Isolations†		
			West Virginia	
		Ohio Samples*	Morganotwn Samples*	Elkins Samples*
Candida utilis	6		U-1.	W-1,S-1,B-1,U-2.
Cryptococcus aeria	11	W-1.	S-2.	W-2,S-2,B-2,U-2.
Cryptococcus albidus var. *albidus*	4	S-1,B-1.	S-1,B-1.	
Cryptococcus albidus var. *diffluens*	3	B-2.	B-1.	
Cryptococcus laurentii	24	W-3,S-3,B-2,U-1.	W-3,U-1.	W-2,S-3,B-2,U-4.
Cryptococcus lutescens	1			S-1.
Kloeckera apiculata	1	B-1.		
Rhodotorula spp.	17	W-1,S-2,B-3,U-1.	W-1,S-2,U-2.	S-3,B-2.
Rhodotorula glutinis	40	W-3,S-5,B-6,U-4.	W-2,S-3,B-2,U-3.	W-3,B-4,S-6,U-1.
Rhodotorula rubra	38	W-3,S-3,B-3,U-3.	W-4,S-4,B-4,U-4.	W-1,S-3,B-4,U-3.
Rhodotorula minuta var. *texensis*	23	W-3,S-1,B-1,U-1.	W-2,B-1.	W-5,S-4,B-5.
Torulopsis spp.	24	S-2,U-3.	W-3,S-4,U-1.	S-5,B-3,U-3.
Torulopsis candida	39	W-2,S-4,B-4,U-2.	W-1,S-4,B-5,U-3.	W-2,S-3,B-5,U-4.
Torulopsis colliculosa	2		S-1.	S-1.
Torulopsis dattila	9	S-1,B-1.	W-2,S-2,B-1.	S-1,B-1.
Torulopsis famata	44	W-2,S-4,B-2,U-1.	W-1,S-3,B-4,U-5.	W-7,S-6,B-5,U-4.
Torulopsis glabrata	17	S-1,U-2.	S-1,B-1.	W-1,S-5,B-4,U-2.
Torulopsis inconspicua	4		S-1.	W-1,S-2.
Torulopsis stellata	11	S-3,B-2,U-2.	B-1,U-1.	S-1,B-1.
Torulopsis versatilis	2	S-1.		S-1.
Trichosporon spp.	7	W-1,B-3.	W-1,B-1.	B-1.
Trichosporon behrendii	1			W-1.
Trichosporon capitatum	1	1		S-2,B-1.
Trichosporon cutaneum	31	S-1,B-2,U-4.	W-3,S-4,B-3,U-1.	W-1,S-5,B-5,U-2.
Trichosporon pullulans	7	W-1.	S-1,B-2,U-1.	S-1,B-1.
Hyphomycetes	1861			
Moniliales				
Moniliaceae				
Geotrichum candidum	58	S-6,B-7,U-5.	W-1,S-7,B-3,U-4.	W-5,S-9,B-6,U-5.
Oidiodendron sp.	1		S-1.	
Arthrinium phaeospermum	3	B-1.		B-1,U-1.
Humicola grisea	2	B-1.	U-1.	
Monosporium apiospermum	1	S-1.		
Sepedonium sp.	2		U-1.	S-1.
Cladosporium spp.	5	W-1,S-1,B-1,U-1.	U-1.	
Cladosporium avellaneum	1	W-1.		
Cladosporium cladosporioides	41	W-4,S-7,B-3,U-6.	W-3,S-4,B-1,U-2.	W-2,S-3,B-2,U-4.
Cladosporium elatum	1	W-1.		
Cladosporium herbarum	16	W-2,B-3,U-1.	W-2,S-2,B-2,U-2.	S-2.
Cladosporium macrocarpum	1			W-1.
Cladosporium sphaerospermum	5	W-1,S-1,U-1.		W-2.
Monilia sp.	1	1		
Costantinella terrestris	2	W-1.	S-1.	
Rhinocladiella mansonii	117	W-10,S-10,B-10,U-12.	W-8,S-8,B-6,U-6.	W-11,S-11,B-8,U-7.
Sporotrichum sp.	1			B-1.
Chrysosporium pannorum	5	W-2,S-1.	U-2.	
Epicoccum purpurrascens	23	W-2,S-2,B-3,U-1.	W-3,S-s,B-1,U-2.	W-2,B-3,U-1.
Doratomyces stemonitis	2	W-2,S-1.		
Isaria farinacea	2	B-1.	B-1.	
Leptographium spp.	9	S-2,B-2.	U-2.	W-1,S-2.
Scopulariopsis sp.	1	U-1.		
Scopulariopsis asperula	2		B-1.	S-1.
Scopulariopsis brumptii	1	B-1.		
Alternaria alternata	27	W-6,S-4,B-4,U-3.	W-2,S-1.	W-4,S-1,B-1,U-1.
Curvularia sp.	1		1	
Curvularia geniculata	4	U-3.	B-1.	
Curvularia lunata	4	W-1,U-1.	S-1,B-1.	
Helminthosporium sp.	1		S-1.	
Stemphylium sp.	1			B-1.
Stemphylium botryosum	1			U-1.

TABLE 1. *Continued.*

Species	Total	No. of Isolations†		
			West Virginia	
		Ohio Samples*	Morgantown Samples*	Alkins Samples*
Torula sp.	2	W-1.	U-1.	
Aureobasidium pullulans	56	W-3,S-6,B-6,U-2.	W-3,S-4,B-4,U-2.	W-10,S-5,B-6,U-5.
Phialocephala sp.	4	S-1,B-1,U-1.		W-1.
Phialocephala dimorphospora	1			B-1.
Phialophora spp.	10	W-2,B-2.	W-1,B-1,U-1.	S-3.
Phialophora sp. S-4-29-4.	3	S-1.	U-1.	S-1.
Phialophora lagerbergii	2			S-1,U-1.
Fusarium spp.	8	S-1,U-2.	S-3,U-1.	B-1.
Fusarium aquaeductuum var. *medium*	9	W-2,S-3,B-1.	W-2.	S-1.
Fusarium moniliforme	1		1	
Fusarium oxysporum var. *oxysporum*	36	W-3,S-2,B-3,U-5.	W-3,S-5,B-3,U-4.	W-2,S-3,B-2,U-1.
Fusarium roseum	11	W-2,B-2,U-2.	U-2.	W-1,B-2.
Fusarium solani	17	W-3,S-1,B-2,U-3.	W-1,S-1,B-1,U-S.	W-1,S-2.
"*Cephalosporium*" sp. PR-1-1-1.	85	W-7,S-7,B-7,U-6.	W-5,S-7,B-6,U-6.	W-11,S-10,B-6,U-7.
"*Cephalosporium*" sp.—PR-2-2-11.	1	B-1.		
Cephalosporium carpogenum	2	S-1,B-1.		
"*Cephalosporium*" *symbioticum*	2	W-1,B-1.		
Acremonium butyri	1			B-1.
Chloridium sp.	1			U-1.
Colletotrichum gloeosporioides	3	W-1,U-1.		S-1.
Gliocladium spp.	12	W-1,S-3,B-1.	S-2,B-1.	S-1,U-3.
Gliocladium catenulatum	15	S-1,B-1,U-3.	W-1,S-1,U-1.	W-1,S-4,U-2.
Gliocladium deliquescens	15	S-2,B-2.	W-3,S-2,B-4,U-1.	B-1.
Gliocladium fimbriatum	9	S-1,B-2,U-2.	W-1,S-2,U-1.	
Gliocladium roseum	41	W-4,S-4,B-6,U-5.	S-5,B-4,U-3.	S-3,B-4,U-3.
Gliomastix murorum var. *felina*	8	W-1,S-2,B-1,U-1.		S-2,U-1.
Myrothecium verrucaria	15	S-1,B-3,U-2.	B-2,U-1.	W-2,S-1,B-1,U-2.
Trichoderma sp.—AM-5-11-4c	1			B-1.
Trichoderma polysporum	10	S-1.	S-2.	S-5,B-2.
Trichoderma viride sensu latissimo	89	W-7,S-11,B-9,U-10.	W-3,S-8,B-5,U-6.	W-4,S-9,B-9,U-8.
Verticillium spp.	10	S-2,B-2,U-2.		S-3,B-1.
Verticillium chlamydosporum	1	S-1.		
Verticillium lateritium	8	W-2.	S-1.	S-2,B-1,U-2.
Pestalotia heterocornis	8	W-1,S-1.	W-1,B-1,U-1.	W-2,B-1.
Aspergillus spp.	21	S-1,B-1,U-1.	W-3,S-2,B-3,U-4.	W-1,S-2,B-3.
Aspergillus chevalieri	4	U-2.	U-1.	B-1.
Aspergillus clavatus	1	S-1.		
Aspergillus fischeri	1	S-1.		
Aspergillus flavipes	1	S-1.		
Aspergillus flavus	37	W-5,S-2,U-4.	W-4,S-6,B-3,U-4.	W-3,S-1,B-3.
Aspergillus fumigatus	47	W-2,S-3,B-1,U-4.	W-3,S-6,B-4,U-6.	W-2,S-6,B-6,U-4.
Aspergillus niger	30	W-4,B-2,U-2.	W-1,S-2,B-2,U-1.	W-6,S-5,B-3,U-2.
Aspergillus ochraceus	12	W-2,S-1,B-3,U-3.	S-1,B-1,U-1.	
Aspergillus restrictus	2	S-1,U-1.		
Aspergillus sydowii	7	S-2,B-1,U-3.		
Aspergillus tamarii	1	U-1.		
Aspergillus terreus	1	U-1.		
Aspergillus ustus	11	W-2,S-2,U-1.	S-2,U-1.	W-1,S-2.
Aspergillus versicolor	13	W-1,S-2,B-2.	W-1,S-3,U-3.	B-1.
Memnoniella echinata	1			W-1.
Paecilomyces spp.	5		B-1.	W-1,S-1,B-1,U-1.
Paecilomyces elegans	1		B-1.	
Paecilomyces marquandii	5	S-1,B-1,U-1.	S-1,U-1.	
Paecilomyces varioti	15	W-1,S-1,B-2,U-2.	W-1,U-1.	S-2,B-2,U-3.
Penicillium spp.	109	W-11,S-10,B-11,U-11.	W-8,S-8,B-6,U-6.	W-9,S-11,B-9,U-9.
Penicillium aculeatum	1			S-1.
Penicillium brefeldianum	3	S-1,B-1.	U-1.	
Penicillium canescens	4		W-1,S-1.	W-1,S-1.
Penicillium charlesii	16	W-1,S-3,B-4,U-5.	S-1.	B-1,U-1.
Penicillium chrysogenum	28	W-1,S-2,B-4,U-2.	S-5,B-4,U-1.	W-1,S-1,U-1.

TABLE 1. *Continued.*

Species	Total	No. of Isolations†		
			West Virginia	
		Ohio Samples	Morgantown Samples*	Elkins Samples*
Penicillium commune	13	S-1.	S-3,U-1.	S-3,B-1,U-4.
Penicillium corymbiferum	7	U-1.	S-2,U-1.	B-1,U-2.
Penicillium crustosum	5	B-1,U-2.		S-1,B-1.
Penicillium cyclopium	1	S-1.		
Penicillium decumbens	3	S-3.		
Penicillium diversum	2			S-1,B-1.
Penicillium expansum	15	S-1.	W-1,S-2,U-1.	W-2,S-5,B-2.
Penicillium frequentens	7	S-1,U-1.	W-1,B-1,U-2.	B-1.
Penicillium funiculosum	14	W-2,S-1,B-3,U-3.	B-1.	S-2,B-2.
Penicillium herquei	3	W-1,U-1.	U-1.	
Penicillium implicatum	7	B-1.	W-1,S-1.	B-3,U-1.
Penicillium islandicum	1		U-1.	
Penicillium janthinellum	58	W-4,S-8,B-6,U-6.	W-3,S-5,B-4,U-5.	W-1,S-7,B-5,U-4.
Penicillium javanicum	30	W-1,S-2,B-2,U-3.	W-2,S-3,B-2,U-4.	W-1,S-2,B-3,U-5.
Penicillium kapuscinskii	6	S-1,B-1,U-1.	B-1.	W-1,B-1.
Penicillium lilacinum	72	W-4,S-7,B-5,U-9.	W-6,S-8,B-6,U-6.	W-6,S-7,B-4,U-4.
Penicillium martensii	28	W-2,B-2.	W-1,S-2,B-1,U-2.	W-2,S-7,B-3,U-6.
Penicillium nigricans	18	W-1,S-3,B-2.	W-1,S-2,B-3,U-3.	B-2,U-1.
Penicillium ochrochloron	40	W-2,S-1,B-1.	W-1,S-2.	W-11,S-9,B-6,U-7.
Penicillium oxalicum	1	S-1.		
Penicillium piscarium	1		S-1.	
Penicillium puberulum	2	W-1,U-1.		
Penicillium pulvillorum	8	W-1.		W-2,S-3,B-1,U-1.
Penicillium purpureogenum	15	W-1,S-1,B-1,U-1.	S-2,B-1,U-1.	W-1,S-3,B-2,U-1.
Penicillium rubrum	6	S-1.	S-1,U-2.	S-2.
Penicillium sclerotiorum	6	W-1,S-2,U-1.	B-1,U-1.	
Penicillium simplicissimum	8	W-2,S-2,B-2,U-1.	S-1.	
Penicillium solitum	8	S-1.	W-1.	S-5,B-1.
Penicillium steckii	3	W-1.	B-1,U-1.	
Penicillium thomii	16	W-1,S-3,B-3,U-1.	S-2.	W-1,B-2,U-3.
Penicillium variabile	12	W-1,S-3,B-4,U-2.	U-1.	S-1.
Penicillium velutinum	1	B-1.		
Penicillium vermiculatum	8		S-3,B-1.	W-1,S-1,B-1,U-1.
Penicillium waksmanii	2		S-1,U-1.	
Periconia sp.	2		S-1,U-1.	
Unidentified stilbaceous fungus	1			B-1.
Unidentified yeast-like fungi	100	W-11,S-12,B-12,U-12.	W-7,S-8,B-6,U-6.	W-6,S-7,B-4,U-9.
Unidentified filamentous fungi	113	W-10,S-11,B-11,U-11.	W-8,S-8,B-7,U-7.	W-11,S-11,B-9,U-9.
Supplement				
Chlorophyceae (Green Algae)				
Prototheca moriformis	1	1		

†Number of locations from which a species was recovered.

*The four major habitat-types sampled at each location are:

W—Stream or lake water;

S—Bottom sediments sampled at arm's length from the shore;

B—Bank soil at interface of stream water and soil;

U—Upland soil above obvious high-water mark of stream.

**sp. refers to incompletely identified species in stated genus; spp. more than one species remains unidentified in stated genus.

***Colonies composed of sterile mycelium bearing clamp connections have been isolated from all Ohio, Morgantown, and Elkins locations. In addition, following culture manipulations, some species of yeasts here included with the Fungi Imperfecti are now considered to be Basidiomycetes.

RESULTS

The species of fungi recovered from these samples are listed in table 1 together with their habitats and the number of locations from which they were recovered in each habitat. Throughout the list there are generic or familial designations without complete identification to species. Where these were from only one sample or station they are noted as

sp.; where from more than one sample or station they are usually noted as spp., in the plural, since more than one species may have been involved. In these cases a number of factors could have been operative. Strains isolated from a sample may not have survived storage until an opportunity to make an identification was available; strains may not have been readily identifiable on the basis of information or monographic treatments available; completed identifications for some strains submitted to specialists have never been made; strains may not have survived holding for submission to experts for identification; or strains may have been lost through careless handling, or in the process of the phasing out of the Fungus Studies Laboratory. In most cases of unidentified strains, few colonies on the primary isolation plates are represented although several appeared, at the time, to be of interest.

The system used in table 1 has been adapted from the several chapters involved in Vols. IV–A and IV–B of "The Fungi, An Advanced Treatise" (Ainsworth, *et al*, 1973). Species in the list of Moniliaceae are arranged after the method of Cooke (1963), without designation of subfamilial groupings. The morphological groups and imperfect spore types are summarized in table 2.

TABLE 2. *Acid Mine-Drainage Fungi by Classes and Morphological Groups.*

BY CLASSES:

	Species	Records
Oomycetes	11	
Zygomycetes	160	
Ascomycetes	21	
Fungi Imperfecti	2530	
Total	2722	

BY MORPHOLOGICAL GROUPS

YEAST-LIKE ORGANISMS

	Species	Records
Ascosporogenous Yeasts	6	21
Basidiomycetous Yeasts	2	
Anascosporogenous Yeasts	37	528
Achlorophyllous alga	1	1
Total	46	550

FILAMENTOUS IMPERFECT FUNGI

Arthric spore producers	2	61
Blastic spore producers	125	1564
Sphaeropsidales	9	150
Undetermined	—	111
Total	136	1886

BY SPORE REPRODUCTION METHODS

FILAMENTOUS FUNGI IMPERFECTI

Category	No. of Species	% of Total	No. of Records	% of Total
Arthric	2	1.6	61	3.8
Blastic				
Phialidic	94	74.0	1291	79.4
All others	31	24.1	273	18.0
Totals	127		1625	

Based on samples collected in Ohio, the numbers of species recovered from the four habitat types at each station, the numbers of species appearing in the three seasons in which samples were taken, and the numbers of records for all habitats in each of the three seasons, are summarized in table 3. It may be noted that stream bottom sediments yielded the larger number of species. More species and records were obtained from summer samples, and fewer in late winter samples, taken when the ground was frozen.

TABLE 3

Habitat and Seasonal Distribution of Species. Based on Ohio samples.

Site	Species	% of Total
SPECIES APPEARING IN ALL SEASONS		
Water	50	37.0
Stream bottom sediments	97	72.0
Bank soil at stream-water interface	91	67.4
Upland soil above usual flood stage	82	60.7
Total species identified for this calculation	135	
NUMBERS OF SPECIES APPEARING IN ALL HABITATS		
Early Spring (3–31–64)	80	59.2
Mid-Autumn (10–9–64)	98	72.6
Late Winter (2–22–65)	62	45.9
SPECIES RECORDS FOR ALL HABITATS IN EACH SEASON		
Early Spring	789	37.0
Mid-Autumn	929	43.7
Late Winter	409	19.2
Total	2127	

DISCUSSION

Fungi are decomposer organisms which are dependent on preformed organic matter. They are neither photosynthetic nor chemosynthetic, and are eucaryotic. Their degradation activities are largely confined to the growing point, and a few micrometers behind it before the tubular wall is thickened beyond the thickness practical for the exchange of ions and molecules through the wall and cell membranes. Exoenzymes capable of degrading nutrients which cannot pass through these membranes are secreted into the environment, and act on organic matter present releasing elements, ions, and molecules which can pass through these membranes thus contributing to the nourishment of the cell and the mycelium behind it. These growing point cells possess exoenzyme systems and secrete enzymes, they also possess endoenzymes, characteristic of individual species of fungi. While some species may be producing carbon dioxide and other terminal byproducts from one set of transformations, other species, may be producing these from another set of transformations. The additional by products in these cases may be quite different giving different residue patterns requiring the activities of different organisms to complete or carry on the transformation series.

The fungial population of moving water is in a state of flux. A population at one point may be adding to the spora flowing downstream which settle at another point. In the benthos, the stream bottom sediments, there may be a greater amount of stability. In the bank soil, even at the interface of the stream water, there is greater stability and in the upland soil there is probably as great stability as in any other soil situation. While from 1 cu mm of soil to another there will be variations in population, there is enough

degree of generalization concerning fungial populations. Within stabilized populations there may be a certain amount of flux through the introduction of species which may displace each other as a result of changes in available nutrients or other factors.

A limited study such as this may not be practical to pinpoint all the above phenomena but a survey of a single area at weekly intervals might develop information which could better describe these phenomena as a result of actual observation rather than theoretical consideration. However, enough work has been done so that generalizations can be made with a fair degree of expectation that they can be demonstrated experimentally.

No attempt was made to correlate possible sanitary sewage or other industrial waste contamination of the small natural streams with acid mine-drainage in an attempt to explain increased or diminished numbers of fungal colonies. It may be noted that excess organic matter, especially under acid conditions, tends to enhance the growth of fungi. It may be assumed that wherever one of these streams passes a home, a farm, or a community, it receives a certain amount of extra organic runoff. Morgantown's raw sewage was being delivered to the Monongahela River, and at least the downstream station could have felt this influence (Wilson *et al*, 1961). The exceedingly acid pond in the abandoned strip mine, used as the Coalton dump, could have received excess organic matter and, indeed this may have been the only organic matter it received, other than leaves from nearby deciduous trees.

Sample sites O–8, O–9, M–3, and E–9, may be considered controls since at the time of sampling no active discharge of acid mine-drainage occurred upstream from them. Site O–8 was on a stream draining farm land at some distance from any coal measures, and site O–9 was on a stream draining territory geologically older than the coal measures in the same county but stratigraphically different, and in a different drainage system. Site M–3 was on a stream 2.1 miles above a coal preparation station above which no coal measures had been opened. Site E–9 was on a stream somewhat removed from coal measures which were being actively worked (See pgs 231–232).

At each sample site small quantities of samples were obtained and subsamples of these were removed for dilution purposes. Thus, a very small portion of the original sample was used for plating and determination of numbers of colonies and kinds of species present in the habitat. Even with 5 replications the size of the finally plated sample was small. In other work it has been shown that larger samples or more replicates would yield little more information on the basis of the law of diminishing returns. Since the whole process was randomized (that is, the original samples were grab samples from which small portions were removed without regard to position in the completely disturbed grab sample, from which smaller samples were removed by pipetting after thorough shaking) it may be assumed that the populations present at any sampled site were adequately represented in the final plates and flasks from which colony counts and species identifications were made. Results from control samples were similar to those in the more heavily polluted samples in each of the three areas, indicating the probability that fungi are not inhibited by acid mine-drainage. The study by Wilson *et al* (1961) showed that at least certain species of fungi may be active in the removal of organic matter under the conditions of relatively high acidity. A study by Cooke *et al* (1956) showed that under conditions of greater acidity, the fungi tested, including strains of some species recovered in this study, are capable of removing biochemical oxygen demand (B.O.D.) producing substances more rapidly, or at least as rapidly, as test organisms present in raw sewage seed.

On the basis of observations made to date, it is difficult to see how the fungi, at least those species listed from these sets of samples, could be involved in the production of acid from iron pyrites or other minerals in the coal measures. Knowledge of acid production by bacteria and oxidation has been summarized by Hanna *et al* (1963). On the basis of the numbers of species and colonies recovered

from the several sites sampled, it is not difficult to see that the fungi are not adversely affected by excessive acid in the stream water, or excessive amounts of soluble or insoluble iron compounds in the water, bottom sediments, or on the stream shore. In fact many fungi, which are capable of adjusting to the habitat, are using whatever available organic matter comes their way in spite of the possible or potential toxicity of acid and iron in the habitat. The habitat and seasonal distribution of 135 species of fungi isolated from Ohio samples are summarized in table 3. It should be noted that while there are differences between the 3 sets of populations observed in this study, the basic groups of species tolerating the habitat in the one Ohio and two West Virginia areas are quite similar. It is suggested that many of the fungi reported in this survey are performing a useful function by removing waste organic matter from the waters of the streams sampled.

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